NOTES

Localization of Translocation Complex Components in Bacillus subtilis: Enrichment of the Signal Recognition Particle Receptor at Early Sporulation Septa‡

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Received 17 December 2004/Accepted 20 April 2005

We here demonstrate that in Bacillus subtilis, the signal recognition particle receptor, FtsY, transiently localizes to early sporulation septa, whereas three SecYEG translocase-associated membrane proteins (SecDF, SpoIIIJ, and YqjG) are uniformly distributed. These results suggest FtsY delivers secreted proteins to SecYEG at the septum, consistent with initial septal localization of forespore membrane proteins.

The mechanism for protein secretion and membrane protein insertion is conserved from bacteria to humans. In bacteria, many exported or integral membrane proteins interact with either the SecA motor or the signal recognition particle (SRP) and its receptor (FtsY) and then transit the membrane utilizing the SecYEG translocase (3, 7, 20). The essential Escherichia coli membrane protein YidC interacts with SecYEG to mediate membrane protein insertion (13–15). Bacillus subtilis and many other gram-positive bacteria encode two YidC homologues, SpoIIIJ and YqjG (21), and B. subtilis requires at least one for viability (8). Interestingly, SpoIIIJ is required for activation of the late sporulation sigma factor, σE (4, 16), suggesting that it and YqjG might comprise specialized membrane protein insertion complexes with unique roles in sporulation and growth.

Localized membrane proteins are crucial for B. subtilis sporulation, participating in signal transduction cascades and morphological processes, such as coat assembly and engulfment (19), but little is known about mechanisms by which proteins localize during sporulation. In the mother cell, membrane proteins appear to reach their correct destination by random insertion, followed by diffusion and capture (12). However, we previously demonstrated that any forespore-expressed membrane protein initially localizes to the septum, suggesting directed insertion into the septum followed by either diffusion or capture (11). Retention of proteins in either cell can be mediated by interactions across the extracellular domains of forespore and mother cell proteins (1). To further investigate the possibility that forespore proteins are directly inserted into the septal membrane domain during B. subtilis sporulation, we localized the translocase-associated proteins SecDF, FtsY (SRP receptor), and SpoIIIJ and YqjG, which were previously reported to localize to sporulation septa (8).

We constructed functional green fluorescent protein (GFP) fusions to the C termini of SpoIIIJ and YqjG and integrated the fusions at the native loci (see the supplemental material), where they provided the only intact copy of the gene. Both GFP fusions are functional, since SpoIIIJ-GFP supported wild-type sporulation and YqjG-GFP supported growth of spoIIIJ mutants (not shown). Localization was monitored during growth and sporulation (9, 11, 17). We noted that YqjG-GFP fluorescence could be observed only in spoIIIJ mutants, suggesting yqjG expression was regulated; indeed, a translational yqjG-lacZ fusion (KP1086) (Fig. 1) was induced ~50-fold in the absence of SpoIIIJ (KP11131), suggesting YqjG substitutes for SpoIIIJ. Both SpoIIIJ-GFP (KP10039) (Fig. 2A) and YqjG-GFP (KP11246) (Fig. 2B) were uniformly distributed during sporulation when expressed from their own promoters or when SpoIIIJ was expressed in the mother cell (KP10068) (Fig. 2C). We quantified GFP and membrane fluorescence at vegetative and sporulation septa, determining the pixel intensity/μm² for >20 cells (averages shown in Fig. 3B) (9, 11). As expected, the ratio of membrane fluorescence at septa versus cytoplasmic or polar membranes was 2:1, since septa contain two membranes (Fig. 3A and B). Identical results were obtained for both SpoIIIJ-GFP and YqjG-GFP (Fig. 3B), confirming that these proteins were not localized to septa before engulfment. After engulfment, the forespore is surrounded by three membranes, and SpoIIIJ-GFP and YqjG-GFP showed fluorescence intensities at the forespore compared to the cytoplasmic membrane of less than 3:1 (quantitation not shown). Thus, both SpoIIIJ and YqjG are randomly distributed throughout the membrane during sporulation, despite previous reports that they localized to septa. These results demonstrate the importance of quantitative analysis and internal controls (such as membrane stains) in protein localization studies, since without quantitation randomly distributed membrane proteins...
will appear enriched at sites of increased membrane layers, such as septa.

We were interested in comparing localization of SpoIIIJ and YqjG to that of Sec translocase components. SecYEG from *E. coli* was recently found to be nonlocalized (2), so we constructed a functional GFP fusion to SecDF, an accessory integral membrane component of the translocase, and monitored localization (KP11271) (Fig. 2D; efforts to construct functional fusions to SecA, SecE, and SecY failed). Again, SecDF-GFP was uniformly distributed throughout the membrane with no enrichment at septa (Fig. 3B), similar to SpoIIIJ and YqjG. We also constructed a functional GFP fusion to FtsY, the SRP receptor protein, and monitored its localization (KP11303) (Fig. 2E, F, G). As predicted by biochemical studies, FtsY-GFP primarily appeared soluble, with strong cytoplasmic fluorescence in all cells (Fig. 2E), and no fluorescence was detected at vegetative septa (Fig. 2E, arrow and arrowhead) (5). Surprisingly, FtsY-GFP localized to invaginating and complete sporulation septa that had not started engulfment (Fig. 2F, arrow). In cells that had initiated engulfment and had curved septa, FtsY-GFP was not localized (Fig. 2G, arrow). Quantitation showed that FtsY-GFP was 15- to 19-fold more intense at flat or invaginating septa and not enriched at vegetative septa (Fig. 3B). Similar sporangia show septal enrichment of forespore-expressed *E. coli* membrane proteins, such as MalF (Fig. 2H, arrow) (11). The enrichment of FtsY at septa raises the
possibility that SRP-dependent proteins might be directly inserted into the membrane at a specific position in the cell: the early sporulation septum. This could explain the initial septal localization of native forespore membrane proteins, such as SpoIIR and SpoIIQ, and also of forespore-expressed MaF, whose membrane insertion depends on SRP in *E. coli* (3). The localization of FtsY to early spore assembly is also in keeping with a role for FtsY in spore coat assembly (6), which commences after septation (10).

This work was supported by the National Science Foundation (NSF 0135955 to K.P. and DBI 0109229 to A.R).

REFERENCES


